Abstract No. misr14

Structures of the GGA3-VHS Domain Complexed With C-terminal Peptides From the Cation-Dependent and Cation-Independent Mannose-6-Phosphate Receptors

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Beamline(s): X9B

Introduction: Mannose-6 phosphate receptors (MPRs) sort their cargo, lysosomal hydrolases, from the trans-golgi network to the late endosomes for subsequent transport to the lysosomes[1]. The correct incorporation of MPR into endosome-bound vesicles is mediated by the so-called GGA proteins. The VHS domains of GGA proteins specifically bind to acidic/dileucine motifs on the cytoplasmic tails of the MPRs with micromolar affinity[2]. This is the first known function for VHS domains from any protein, and VHS domains are the first definitively characterized binding partners for acidic/dileucine motifs. We wanted to determine the specific determinants on the GGA VHS domains responsible for binding acidic/dileucine motifs.

Methods and Materials: Crystals of selenomethionylated VHS domain from human GGA3 complexed respectively with peptides derived from the C-termini of the cation-independent and cation-dependent MPRs were grown and brought to the beamline. They were cryprotected on site by quick soaking in crystallization buffer supplemented with glycerol. Two 3-wavelength MAD data sets were collected at the Selenium edge. Data was reduced on site. Structure solution was carried out at the home facility.

Results: The structures of GGA3-VHS complexed with the MPR C-termini were solved successfully. The all-helical VHS-domain binds the peptides in an extended conformation, so that the peptides lie in a groove between two parallel helices of the domain. The acidic and dileucine motif on the peptides interact with a positively charged cluster of residues and two hydrophobic pockets respectively. Several interactions with the peptide backbone add to specificity.

Acknowledgments: We gratefully acknowledge the assistance of the beamline personnel (U. Ramagopal, K.R. Rajashankar) and our laboratory colleagues (R.Trievel, E.Jones) with data collection and processing.

References:

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